FIELD ANALYSIS OF MICROBIAL CONTAMINATION USING THREE MOLECULAR METHODS IN PARALLEL H. Morris[1], E. Stimpson[2], A. Schenk[1], A. Kish[3], M. Damon[4], L. Monaco[1], N. Wainwright[2], and A. Steele[3].

[1]Jacobs ESTS Group, Hunsville, AL 35806, [2]Charles River Laboratories, Charleston, SC 29407, [3]Carnegie Institution of Washington, Washington, DC 20015, [4]BAE Systems, Huntsville AL 35806

Introduction: Advanced technologies with the capability of detecting microbial contamination remain an integral tool for the next stage of space agency proposed exploration missions. To maintain a clean, operational spacecraft environment with minimal potential for forward contamination, such technology is a necessity, particularly, the ability to analyze samples near the point of collection and in real-time both for conducting biological scientific experiments and for performing routine monitoring operations. Multiple molecular methods for detecting microbial contamination are available, but many are either too large or not validated for use on spacecraft. Two methods, the adenosine-triphosphate (ATP) and *Limulus* Amebocyte Lysate (LAL) assays have been approved by the NASA Planetary Protection Office for the assessment of microbial contamination on spacecraft surfaces [1]. We present the first parallel field analysis of microbial contamination pre- and post-cleaning using these two methods as well as universal primer-based polymerase chain reaction (PCR).

Methods: In order to assess microbial contamination using the LAL assay, we utilized the Lab-On-a-Chip Portable Test System (LOCAD-PTS), which consists of a handheld device that has been successfully tested onboard the International Space Station [2]. Cartridges lined with four distinct capillary channels house reagents required to react with any lipopolysaccharide (a Gram-negative bacterial cell wall molecule) present in the sample, culminating in the release of a dye that is quantified using a 395 nm filter. The LAL assay detects the Gram-negative bacterial cell wall molecule, endotoxin or lipopolysaccharide (LPS). The more dye released, the greater the concentration of endotoxin in the sample. Sampling, quantitative analysis, and data retrieval require less than 20 minutes. This timeframe is similar to the analysis time of the ATP assay, which is based on the quantification of the luminescence of the sample. Free ATP, which is predominantly found in living cells but not restricted to microbes, reacts with the luciferase enzyme to produce a luminescent signal. This signal can then be quantified using a standard curve. Taken together, the ATP and LAL assay data provide a more comprehensive analysis of the Gram negative microbial bioburden coupled with a quantification of live cells on a surface.

Effective, yet rapid cleaning protocols are integral to the successful prevention of forward contamination by manned and unmanned exploration missions. Previous field experiments described an effective protocol for removing virtually all biological molecules [3], and a modification of this protocol was used to clean select equipment surfaces between sample collections.

Results and Conclusion: We present data describing the results from the analysis of microbial contamination on the surface of a Mars rover mock-up using three advanced technologies in parallel. These data were collected during the Arctic Mars Analogue Svalbard Expedition (AMASE 2009) to the Arctic in August 2009 and represent the first field analysis using three molecular assays in parallel. The LAL assay, ATP assay, and PCR all demonstrated detection of contaminating organisms on the surface of the Mars rover prior to cleaning. While the samples collected prior to cleaning had to be diluted 100-fold to be analyzed within the range of the PTS unit, the samples from the cleaned surface yielded results that were barely above background. The ATP assay results demonstrated a marked reduction in relative light units (RLUs) after cleaning, from 9.83E+03 to 2.00E+01 for one sample. The PCR data indicated the presence of contaminating bacteria in all samples and eukaryotic cell contamination in one sample prior to cleaning, and detected no pro- or eukaryotic contamination on any surface after cleaning. Collectively, these data demonstrate that all of the advanced technologies investigated provided similar results prior to and following cleaning using a stringent cleaning protocol. Not only do these data verify the stringency of the cleaning protocol using three distinct methods, but these data also could determine future cleaning protocols used prior to or during future exploration missions.

References: [1] NASA Technical Handbook. (2008) *Handbook for the Microbial Examination of Space Hardware.* NASA-HDBK-6022. [2] Maule, J. et. Al. (2009) *Astrobiology, 9(8),* 759-775. [3] Eigenbrode, J. et al. (2009) *Astrobiology 9(5),* 455-465 .

Field Analysis of Microbial Contamination Using Three Molecular Methods in Parallel

H Morris[1], E Stimpson[2], A Schenk[1], A Kish[3], M Damon[4], L Monaco[1], A Steele[3], and N. Wainwright[2]

[1]Jacobs ESTS Group [2]Charles River Laboratories [3]Carnegie Institution of Washington [4]BAE Systems

Introduction

Advanced technologies with the capability of detecting microbial contamination remain integral tools for answering science questions on orbit and verifying the cleanliness of sampling equipment. The ability to analyze samples near the point of collection and in real time is particularly desirable for both applications. We present the first parallel field analysis of surface microbial contamination both pre- and post-cleaning utilizing the Limulus Amebocyte Lysate (LAL) assay and adenosine tri-phosphate (ATP) analysis as well as a universal primer-based polymerase chain reaction (PCR) assay for DNA profiling.

Figure 1. Sampling the rover claw surface while participating in the field exercises of the Arctic Mars Analogue Svalbard Expedition (AMASE 2009).

Objectives

- Obtain reproducible field data on levels of microbial contamination using three complementary molecular biology techniques.
- Verify the efficacy of the cleaning protocol in reducing/eliminating microbial molecules from different rover surfaces.

Methods

- Use sterile swab, collect a sample from the rover surface before cleaning, and a sample after cleaning.
- Split samples to analyze for the presence of ATP, endotoxin, and eukaryotic, prokaryotic, archael DNA.

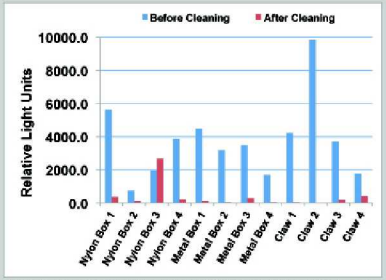

Figure 2. Data acquired from three different surface materials on the rover before and after cleaning. Samples were analyzed using a Pallchek ATP luminometer and values are given in relative light units, with blanks for each sample subtracted.

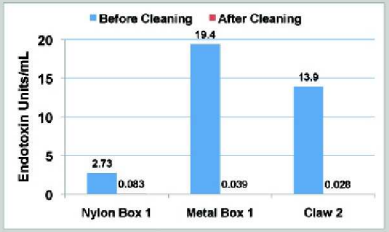

Figure 3. LAL assay data from the same three surface materials before and after cleaning.

	Sample ID	ATP RLU	LAL Sample EU/mL	Bacterial Universal Primers Result	Eukaryotic Universal Primers Result	Archaeal Universal Primers Result
Pre-Cleaning	Nylon Box 1	5630.0	2.73	-	-	-
	Metal Box 1	4490.0	19.4	-	-	-
	Claw 2	9830.0	13.9	-	+	-
After Cleaning	Sample ID	ATP RLU	LAL Sample EU/mL	Bacterial Universal Primers Result	Eukaryotic Universal Primers Result	Archaeal Universal Primers Result
	Nylon Box 1	351.0	0.083	-	-	-
	Metal Box 1	110.0	0.039	-	-	-
	Claw 2	20.0	0.028	-	-	-

Table 1. Direct comparison of all samples from three rover surfaces using all three molecular methods before and after cleaning.

Results and Discussion

- All three assays detected contaminating microbial compounds on the surface of the Mars rover prior to cleaning.
- Samples collected from surfaces cleaned using a multi-step cleaning protocol demonstrated a significant reduction in signal from all three assays. Verifies the efficacy of the cleaning protocol.
- Enhanced optics and the modified cartridge format allow visualization of a multi-feature protein microarray.
- Presents a format for the validation of cleaning protocols and the assessment of microbial contamination of instruments prior to conducting any scientific analyses.

Acknowledgments

This research and technology development effort is made possible by funding by the NASA Exploration Systems Mission Directorate and the Science Mission Directorate. Special thanks to the FIDO Mars Rover team at JPL for allowing us to swab their equipment, to Dr. Mary Voytek for sample handling and storage, and to the AMASE team for organizing the field expedition.